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Effect of phytoestrogen intake in the diet on lipid and sex hormone metabolism in menopausal women, and increased risk of breast cancer

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Abstract

Introduction and objective. Phytoestrogens are one of nutritional factors exhibiting a chemoprotective action, potentially reducing vasomotor symptoms as well as cancerous lesions. The study was ocused on an association between the consumption of phytoestrogens in the diet, blood serum cholesterol concentration and urinary estrogen metabolites levels.
Materials and methods. The study was conducted on 55 women in the perimenopausal period, who were characterized by an increased risk of hormone-dependent cancer. Energy and nutritive value of food rations, and especially the level of phytoestrogen consumption in the diets in the examined population, were analyzed using the Wikt Pro computer programme. Estrogen metabolites (2OHE, 16α-OHE1) were determined from 24-urine samples by the ELISA method. The EMR index was calculated. Plasma total cholesterol concentrations was measured.
Results and conclusions. The population with a higher consumption of phytoestrogens and dietary fibre in the diet, at the simultaneous slight limitation of energy value of the food ration, is characterized by an improved blood serum cholesterol concentration and an appropriate estrogen metabolism towards a significant increase in the estrogen index for the cancer lesions in the mammary glands.

Key words

phytoestrogens, hormone-dependent cancers, nutrition habits, lipids and hormonal metabolism

INTRODUCTION

Reduced hormonal efficiency of the ovaries in the perimenopausal period, apart from dysmenorrhoea, causes several early and late clinical symptoms which are connected with the insufficient antiatherogenic, antioxidant effect of estrogens and the ineffective normalization of lipid metabolism. At the same time in menopausal women, there can frequently be observed an increase in body weight, which additionally increases the risk of the above mentioned dysfunctions.

It is believed that extended menopausal hypoestrogenemia is one of the significant factors responsible for the development of atherosclerosis, its cardio-vascular complications, or osteoporosis. Moreover, estrogens may directly influence proliferation processes, differentiation and gene expression, and have an indirect effect through carcinogenic metabolites. Estrogen metabolites are activated by the cytochrome P450 complex and as reactive forms may cause DNA damage, exhibiting procarcinogenic action (e.g. 16α-hydroxyestradiol), although certain of them show anticancer properties, e.g. 2-methoxyestradiol. In view of the fact that following menopause peripheral tissues are the basic sources of estrogens, local concentrations of hormones in those places may be higher. In the case of mammary glands, this situation causes an adverse effect in the form of an increased risk of cancer development [1, 2, 3].

One of nutritional factors exhibiting a chemoprotective action, potentially having a beneficial effect on the organisms of menopausal women, is connected with compounds of plant origin with a hydroxyphenolic structure, i.e. phytoestrogens. Numerous studies indicate that they have a positive action reducing vasomotor symptoms [4, 5, 6] as well as cancerous lesions [7, 8, 9, 10, 11, 12].

Antioxidant properties of phytoestrogens have been found both *in vitro and in vivo* [13]. At the same time, their role in cell protection against lipid peroxidation and modulation of synthesis of prostaglandins and leukotrienes participating in the process of carcinogenesis [14, 15], inhibition of topoisomerase activity and angiogenesis, seem to constitute important mechanisms, thanks to which they may contribute to a reduced incidence of cancer [16]. In view of the fact that it is necessary to estimate the amount of an effective dose of these compounds in the food ration, the aim of this study was to assess phytoestrogen intake in the diet of menopausal women at risk of breast cancer, as well as evaluate their effect on lipid metabolism and estrogen metabolism.

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MATERIALS AND METHOD

The study was conducted on 55 women in the perimenopausal period (n=55; age 45-55 years, BMI 24.5±3.8), who were characterized by an increased risk of hormone-dependent cancer, i.e. women with the BRCA1 gene mutation, women with a family history of such cancer, as well as women in which breast cancer had been diagnosed previously. The analyzed population of women was divided in terms of the mean daily intake of total phytoestrogens: women with a low level of phytoestrogens in the diet (KNF) were supplied less than 600 μg phytoestrogens daily, patients with a medium level of phytoestrogen uptake (KSF) consumed 600 - 1000 μg a day, while women with a high phytoestrogen level in their daily food rations (KWF) had an uptake of these compounds of over 1,000 μg a day.

The level of phytoestrogen consumption in the diets of the examined women was estimated based on the electronic data base of phytoestrogen levels in foodstuffs and dishes in the Wikt Pro diet software. The data base for the assessment of phytoestrogen levels in the food ration was developed using American and British data concerning contents of individual phytoestrogen groups in foodstuffs [17, 18, 19], and modified in view of the specific character and variety of foodstuffs available in Poland. Assessment of phytoestrogen consumption included intake of total phytoestrogens, isoflavones, lignans, SECO, MAT and coumesterol. Moreover, the structure and frequency of consumption were analyzed for products with varying phytoestrogen contents, distinguishing group A of products with a low phytoestrogen content (below 100 μg/100 g product), group B with a medium phytoestrogen content, which included products containing 100 - 500 μg/100 g, while products constituting the richest source of phytoestrogens (group C) comprised those containing over 500 μg phytoestrogens/100 g of the product.

The energy and nutritive value of food rations in the examined population were analyzed also using the Wikt Pro computer programme, and referred to nutrition standards developed by the Institute of Food and Nutrition in Warsaw [20] for women in the age groups 31–50 years and 51–65 years, with a mean body weight of 45–80 kg and a low level of physical activity (PAL=1.5).

Moreover, in this study, total blood serum cholesterol level was determined in accordance with the standard procedure and the level of estrogen metabolites in urine assessed. 24-h urine was collected into 2.5-L jars, each containing 2 g of ascorbic acid to preserve the estrogen metabolites. Subjects refrigerated the collected urine until it was processed. Samples were stored at –20 °C until all subjects completed the study. The 3 daily collections were then proportionally pooled into a single 72-h sample before analysis. The concentration of estrogen metabolites in each sample pool was multiplied by the mean 24-h urine volume for each 3-d pool, and data were analyzed and expressed as μg of metabolite/24h. Estrogen metabolites 2-OHE (2-hydroxyestrogen) and 16α-OHE (16α-hydroxyestrone) were analyzed in urine by enzyme immunoassay (Estramet 2:16 Enzyme Immunoassay Kit, Immuna Care), as previously described [21, 22]. Each sample was assayed in triplicate and the mean was used. All 5 samples from each subject were run on a single plate, and the same kit lot was used for all study samples. Samples falling below the assay detection limits were assigned the value of the lowest standard. Interassay CVs were 6.5 and 10% and intra-assay CVs were 2% and 3% for 2OHE and 16OHE1, respectively. The concentration of estrogen metabolites in each sample pool was multiplied by the mean 24-h urine volume for each 3-d pool, and the data analyzed and expressed as μg of metabolite/24 h. The estrogen index 2:16OHE1 [EMR] was calculated [21].

The experimental data were subjected to a statistical analysis using one-way ANOVA and Kruskal-Wallis test.

RESULTS AND DISCUSSION

In the presented study, selected nutritional parameters and biochemical indexes in blood serum and urine were determined, taking into consideration the diverse intake of phytoestrogens in the daily food ration of menopausal women at risk of hormone-dependent mammary gland cancer [Tab. 1]. In the great majority of women, a too low CRP energy uptake was recorded, which did not correspond to a higher BMI, indicating overweight or obesity in those women, which probably resulted from a purposeful reduction of calorie content of the diets by the patients, or inaccurate records in the nutrition interviews. It was shown that respondents of nutrition studies typically underestimate data concerning the level of product consumption [23, 24]. Assessment of energy and nutritive value of the diets showed that the KNF group of women consuming foods with a high energy value (2,107±175 kcal/24h), including the highest percentage of fat in the diet (38%), had the lowest intake of phytoestrogens – on average 404±153 μg/day, while in the KSF and KWF groups of women having a comparable energy value, 1,757±331 kcal/24h vs. 1,916±295 kcal/24h, respectively, in which the percentage of fat was 32–34%, phytoestrogen intake was significantly ($p<0.001$) higher (KSF

Table 1. Characteristics of selected nutritional and biochemical parameters of the analyzed population of women depending on phytoestrogen intake in daily food ration

Parameter	Population of women – phytoestrogen intake in diet			Statistical analysis
	KNF [n=10]	KSF [n=17]	KWF [n=8]	
	Nutritional parameters [mean ± SD]			
Energy [kcal/24h]	2107 ± 175 ^{A}	1757 ± 331 ^{B}	1916 ± 295 ^{A}	$p<0.01$
Total phytoestrogens [μg/day]	404 ± 153 ^{A}	781 ± 135 ^{B}	1444 ± 388 ^{C}	$p<0.001$
Cholesterol [mg/day]	265 ± 90	247 ± 81	235 ± 65	NS
UFA [g/day]	31.5 ± 11.5 ^{A}	23.1 ± 5.4 ^{B}	19.3 ± 7.1 ^{B}	p=0.03
Dietary fibre [g/day]	12.6 ± 2.2 ^{A}	16.1 ± 4.3 ^{A}	21.8 ± 4.9 ^{B}	p=0.01
	Biochemical parameters			
Blood cholesterol level [mg/dl]	248 ± 25	215 ± 22	221 ± 40	NS
2-OHE [ng/ml]	13.2 ± 4.2 ^{A}	12.6 ± 3.5 ^{A}	14.9 ± 2.6 ^{B}	p=0.06
16α-OHE [ng/ml]	8.8 ± 3.4 ^{A}	9.0 ± 2.6 ^{A}	7.7 ± 1.8 ^{B}	$p<0.05$
Estrogen Index [2:16α-OHE]	1.5 ± 0.4 ^{A}	1.4 ± 0.6 ^{A}	1.9 ± 0.7 ^{B}	$p<0.05$

^{AA} – No significant differences observed
^{AB} – Significant differences observed p≤

781±135 µg/day; KWF 1,444±388 µg/day). The high energy intake in the diet corresponded with a higher amount of saturated fatty acids SFA in the KNF group with the lowest phytoestrogen intake in the food ration (31.5±11.5 g/day) in relation to the population of women with a medium and high phytoestrogen content in the diet (KSF 23.1±5.4 g/day; KWF 19.3±7.1 g/day), with the differences being significant at p=0.03. The inappropriately balanced nutrient intake, including mainly the high energy ration coming from fats, are fully confirmed in a study by Goluch-Koniuszy et al. [25]. According to Prentice R. L. et al. [26] it needs to be stressed that the low fat consumption at 20% energy supply drastically reduces the risk of development of hormone-dependent cancers. A detailed analysis of the volume and structure of phytoestrogen, supply as well as their sources in the diets of the population of the Polish women, turned out to be comparable to the amount of these compounds supplied in the food rations in other European countries. In Spain, the assessed phytoestrogen intake in food rations of perimenopausal women was, on average, 890 µg/day [27], while in Germany it was 940–962 µg/day [28]. The performed evaluation of diets in terms of phytoestrogen intake did not bring a spectacular increase in the level of dietary fiber, the amount of which in the diet of the examined women was comparable with the level recorded for menopausal women in a study by Ilow et al. [23], while the increase in plant origin products with an increased amount of phytoestrogens led to values reported by Goluch-Koniuszy et al. [25]. At the same time, the amount of cholesterol in the diet of the investigated population did not exceed admissible values, and was slightly higher in women characterized by the lowest phytoestrogen level in the diet (KNF 265±90 mg/day; KSF 247±81 mg/day; KWF 235±60 mg/day).

The nutrition model in the analyzed population of women with an increased supply of phytoestrogens in the diet was reflected in the profile of estrogen metabolites in urine and the level of the estrogen index EMR, while their values fell within reference ranges. Analysis of metabolite levels showed that the groups of women characterized by low (KNF) and medium (KSF) levels of phytoestrogens in daily food rations, in comparison to the group with high phytoestrogen content (KWF), on average, had significantly ($p<0.05$) higher levels of estrogen promoting the cancer process 16α-OHE (8.8±3.4 ng/ml; 9.0±2.6 ng/ml vs. 7.7±1.8 ng/ml), at the simultaneous trend ($p=0.06$) to a slightly lower value of the metabolite inhibiting carcinogenesis within the mammary gland 2-OHE (KNF 13.2±4.2 ng/ml; KSF 12.6±3.5 ng/ml vs. KWF 14.9±2.6 ng/ml). Moreover, it was observed that with an increase in the level of phytoestrogen consumption in the diet of the analyzed population in women at risk of breast cancer in the perimenopausal period, a significant ($p<0.05$) increase was recorded in the estrogen index EMR (KNF 1.5±0.4; KSF 1.4±0.6 vs. KWF 1.9±0.7).

In the conducted investigations, it was found that an increase in the supply of total phytoestrogens in the food rations of the analyzed women corresponded to the simultaneous changes in the structure of urinary estrogen metabolites levels [Fig. 1]. An increase in the amount of lignans was significantly correlated with an increase in the level of the metabolite inhibiting the process of carcinogenesis within the mammary gland 2-OHE, while an increase in the supply of isoflavones was reflected in a decrease of the level of estrogen enhancing the cancer process 16α-OHE.

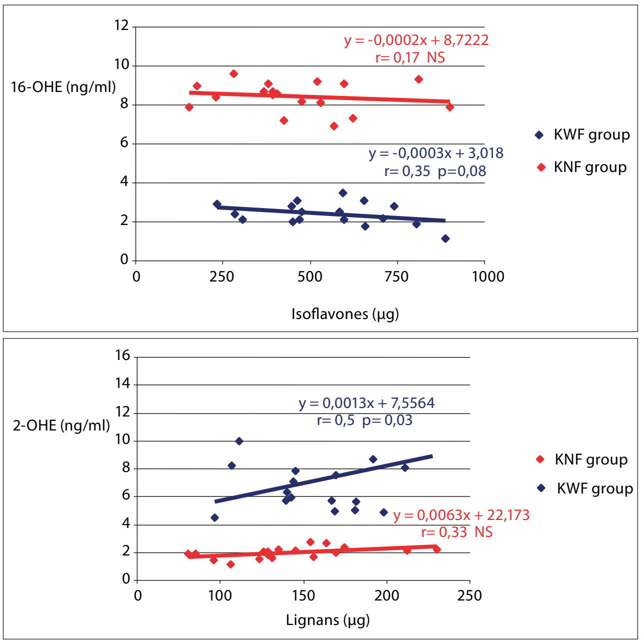

Figure 1. Relationship between classes of phytoestrogen and urinary estrogen metabolites levels

Among the different types of phytoestrogens, the greatest intake level was found for isoflavones, and was less marked for lignans, while it may be assumed that the consumption of these compounds in Poland is relatively high, since for comparison, the estimated intake of isoflavones in Spain was only 0.12 mg [8], in Germany – 0.28–0.29 mg [28], while in the UK it was 0.30–0.63 mg daily [26]. At the same time, the consumption of lignans among Spanish women was much higher and amounted, on average, to 1.32 mg [27], while in the population of Chinese women the intake reached the maximum level of 1.9 mg [29].

CONCLUSIONS

Taking into consideration the nutrition model for women in the perimenopausal period, with a family history or genetically at risk of hormone-dependent breast cancer, it was observed that the population with a higher consumption of phytoestrogens and dietary fibre in the diet, at the simultaneous slight limitation of energy value of the food ration, is characterized by an improved blood serum cholesterol concentration and an appropriate estrogen metabolism towards a significant increase in the estrogen index for the cancer lesions in the mammary glands.

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